THE DEVELOPMENTAL MORPHOLOGY OF THE POLLEN OF PROTEACEAE.

By S. Garside.

Over a long period of years, the pollen grains of Gymnosperms and Angiosperms have been studied entirely from a morphological standpoint, most of the researches into their development and physiology having been made comparatively recently. A comprehensive historical review, more especially of the morphological work, has been given by Wodehouse (1935, pp. 16–100).

Most of the earlier workers were concerned with purely descriptive accounts of the mature grains, or with their comparative morphology, and it remained for Wodehouse (1935, 1936) to put forward a general theory of the evolutionary relationships of pollen forms in Gymnosperms, Monocotyledons and Dicotyledons.

Whilst this clever theory has many ramifications, its more essential features are that the monocolpate or one-furrowed grain which is so prevalent in Monocotyledons and a few of the primitive Dicotyledons, is the direct homologue of the monocolpate grain found in many Gymnosperms, whilst the tricolpate, or three-furrowed grain so prevalent as the basic form in Dicotyledons is a new development, the three furrows in this case being meridional, at right angles to the spore equator, and alternating with the triradiate marking of the inner or proximal face of the spore when still in the tetrad. (Fig. 15.)

In the one-furrowed grain of Monocotyledons, the furrow is said to be formed on what is the distal or outer face of the spore in the original spore tetrad. In pollen without furrows, but with one germ-pore only (as in grasses) or with three germ-pores (as in some Dicotyledons) the pores are regarded as modifications of the furrows and occupy similar positions in each case.

According to this theory then, the great evolutionary advance made by the Dicotyledons is in the development of three equatorial furrows or pores, resulting in the possibility of pleurosiphonic germination, that is of several possible places of exit for the pollen-tube, situated on the equator, as opposed to basisiphonic germination, or germination from one pore only (or furrow) situated on the outer or distal face of the grain.

Wodehouse has quoted very few researches into the development of the mature pollen grain from the spore tetrad stage, especially researches in which the relative positions of the furrows or pores have actually been observed, and indeed this could scarcely have been expected of him, as very little really precise knowledge of this kind exists.

He has therefore had to rely chiefly upon evidence obtained from the positions of furrows or pores in such grains as retain the tetrad grouping until maturity, e.g. Drimys with one pore on the distal face of each grain of the tetrad, and Erica with pores in six pairs. (Fig. 15.)

Cytologists usually lose interest in the pollen grain during the period between the completion of tetrad division and germination, consequently very few workers trouble to figure the changes undergone by the cell walls of the grains during their maturation, although in most cases adequate material for the purpose must have been available.

Thus a great deal of information of use to the pollen morphologist is not forthcoming, and lack of researches of this nature is doubtless the reason why Wodehouse has been forced to make so many assumptions (e.g. Wodehouse, 1935, pp. 334, 336 and 338) in support of his theory.

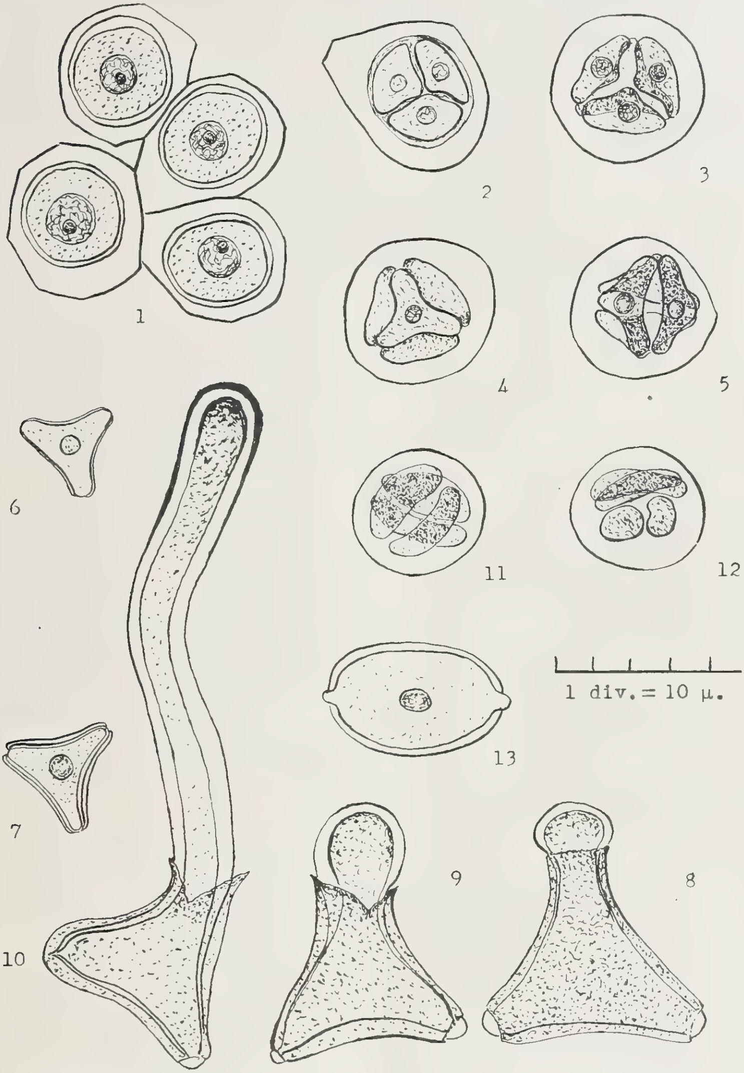
Doubt has already been cast upon the assumption that all monocolpate grains are homologous, e.g. the monocolpate grains of *Asimia angustifolia* A. Gray (Anonaceae) have been shown by Golub to have the furrow on what is the proximal or inner face of the spore when in the tetrad (Bailey and Nast, 1943) and it is now obvious that investigation of almost every case will be necessary before exact homologies can be elucidated.

The present work was undertaken in order to determine the relative positions of the germ-pores in the tetrad resulting from the division of the spore mother cell in Proteaceae ; only the gross developmental features of the cells concerned were examined, cytological details being neglected.

From an extensive series of measurements of numerous genera and species of South African Proteaceae, the writer found that *Leucadendron argenteum* R.Br. has the largest mature pollen grain (45μ to 61μ in diameter) and this plant was therefore chosen for investigation as it was likely to have a large spore tetrad. In addition, it has three large circular germ-pores (11·5μ to 12μ in diameter) one at each angle of the flattened triangular grain. (Fig. 8.)

Tetrad division stages in *L. argenteum* are to be found in the anthers in June, when the young spherical knob-like spike of the male inflorescence is only about 1 cm. in diameter. Usually a developmental series can be found in a single inflorescence, as the lower flowers of the spike are considerably more developed than the upper ones.

Material preserved in 75% alcohol was found adequate for all purposes ; from it the spore tetrads can be dissected out entire and moved about and rotated under the microscope to obtain various views of the same



FIGS. 1—10. *Leucadendron argenteum* R.Br. 1. Spore mother cells. 2, 3, 4, and 5. Tetrahedral spore tetrads. 6. Young microspore immediately after liberation from tetrad. 7. Young pollen grain, showing differentiation of exposporium. 8 and 9. Pollen grains showing early stages of germination. 10. Germinating pollen grain with pollen-tube. FIGS. 11—13. *Embothrium coccineum* Forst. 11 and 12. Decussate tetrads from two points of view. 13. An almost mature pollen-grain. The scale is for all the figures.

tetrad. Microtome sections were not made, and no staining was necessary, though light staining with acetic methyl-green was of value. All the drawings have been made to the same scale so that the relative sizes of the various stages can be seen at a glance. The germination studies were of course made on fresh material.

From the younger anthers of the above-mentioned material, it is easy to isolate groups of spore mother cells (Fig. 1) when each cell is seen to be surrounded by a broad, clear gelatinous zone. In anthers in which tetrad division has taken place (Figs. 2 and 3) the spore mother cells have separated and the gelatinous wall becomes rounded off (Figs. 3, 4 and 5) and the tetrads are completely isolated. The young spores which compose the tetrad always have the tetrahedral arrangement and no deviation from this was observed. Various views of tetrads are shown in Figs. 3, 4 and 5; Fig. 5 is of special interest as at first glance it would appear to be an example of the decussate arrangement, but by revolving such a tetrad under the cover-glass, a view similar to that in Fig. 4 will appear. It is, in fact, only a somewhat unfamiliar view of the tetrahedral arrangement.

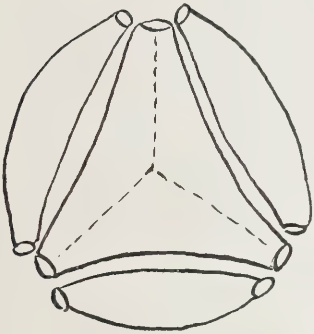
The clear gelatinous wall surrounding the spore tetrad now dissolves, and the spores readily separate (Fig. 6) without other change than that of rapid enlargement and thickening of the spore wall. When the exospore and endospore are differentiated (Fig. 7) it can easily be observed that the germ-pores are present on the angular tips of the spore (Fig. 7), i.e., they are on the spore equator and terminate the lines of the tri-radiate marking, which does not, of course, persist in the mature spore. Fig. 14 gives a diagram of the spores in the tetrad to illustrate the position of the pores in a spore in relation to the tri-radiate marking, and also to show the grouping of the pores in four groups of three, one group of three not being visible in the figure.

Fig. 14 should be compared with Fig. 15, which represents a tetrad of Erica, in which the germ-pores are seen to be alternating with the tri-radiate marking, and are grouped in six groups of two, an arrangement which Wodehouse appears to have considered to be typical of all tricolpate and three-pored grains in Dicotyledons.

It is clear therefore, that most of the tricolpate and three-pored pollens will require investigation before we know their real nature, and following the type of nomenclature used for embryo-sac types, we may call them after the genera or families in which they were first discovered.

The type shown in Fig. 15 has been established for Epilobium by a study of its development by Luerson (quoted by Wodehouse, 1935, p. 91) and might be called the "Epilobium type," whilst the development which follows Fig. 14 could be called the "Proteaceous type." Such a terminology will only be of value until a wide survey of developmental types has been made, when we may expect the types to fall into some logical sequence, and it will then no longer be necessary.

In the writer's opinion, the proteaceous type may represent a very primitive spore type in Dicotyledons, as the grain has become very little altered from its original tetrahedral shape given to it in the tetrad division, and in this respect resembles the spores of many liverworts and of Sphagnum and some ferns. The question then arises as to the germ-pore in the proteaceous type being more primitive than the fold or colpa frequently found in spherical pollens. The fold or colpa is, as Wodehouse has clearly indicated, primarily an organ of size adjustment to the water content of the grain, and appears to be a decided advance on the simpler pollen of Proteaceae which has no such obvious specialisation. In any event, it is clear that the fold in many pollens is, because of its position, not the homologue of the pore in proteaceous pollen.



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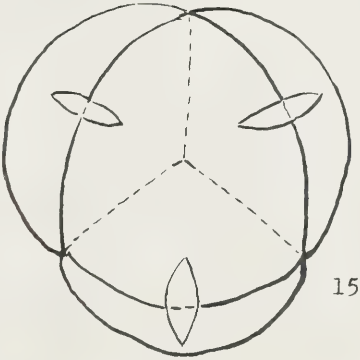

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Fig. 14. Tetrahedral pollen tetrad of Proteaceae.
Fig. 15. Pollen tetrad of Erica. Both figures are diagrammatic.

It is noteworthy that Thompson in his brief treatment of pollen grains (Thompson, 1942) remarks that the three folds "are formed (I take it) along lines of weakness at the edges of the tetrahedron," and it may be assumed that they would be more likely to form in grains in which an early thickening of the exosporium (preserving the tetrahedral form) does not occur. However, in making any assumptions regarding evolution, we must bear in mind Thompson's warning (loc. cit., p. 613) that resemblances in spores are "a matter of physical and mathematical symmetry, and carry no proof of near relationship or common ancestry."

GERMINATION.

As the writer's object was to observe germination, and not to investigate the physiological conditions necessary for it, only a few pollen cultures were made.

Germination was slow in all cases, and comparatively few grains in each culture showed the first stages of germination, and fewer still formed a long pollen tube. 50, 20, 10 and 5% cane sugar solutions in water were used, the lower concentrations giving the best results.

No doubt the addition of ripe stigmas, yeast or traces of mineral salts might have greatly stimulated germination, but these experiments remain for future workers.

From the morphological viewpoint, it is to be noted that not more than one tube was ever obtained from one grain, and that there is no " dehiscence " or splitting away of the exospore on germination, only occasionally a slight tearing or cracking of the margins of the pore from which a tube has emerged, is to be observed. (Figs. 9 and 10.)

However, a very remarkable feature was the extreme thickness of the cellulose wall of the pollen tube (Fig. 10) which may be up to 5μ thick ; a feature which has also been noticed by the present writer in other proteaceous pollen tubes, e.g., *Grevillea oleoides* Sieb., where the tube is very irregularly thickened. Tassi (1898) has also figured the pollen tube of Stenocarpus as having fairly thick walls.

In concluding this account of the three-pored pollen of *Leucadendron argenteum*, we must make a brief reference to the work of Brough (1933), who has investigated the development of the microspores of *Grevillea robusta* Cunn. After describing the miscospores as of " a precise tetrahedral form in the nearly mature flower bud " (loc. cit., p. 51), he says that the " thickening of the exine is not uniform however, and is distributed in such a manner that four unthickened areas are evident at the points of the tetrahedron," and also says that "the consequent increase in internal pressure is no doubt the cause of the protrusion of the intine at the four corners of the tetrahedron." These expressions undoubtedly lead one to suppose that there are here four germ-pores, and the writer has therefore re-examined the pollen of *G. robusta*, and finds it to be of the normal three-pored type. Brough's error is probably due to a *lapsus calami*, as his figures of the microspores all show three pores only.

BIPOROSE POLLEN.

We must now briefly consider the case of proteaceous pollen in which the grain is ovoid or sausage-shaped, and in the latter case often somewhat arcuate, with one pore at each end.

These peculiar two-pored grains are known for the genera Banksia, Dryandra and Embothrium, in which this form of pollen is a generic character in each case ; no examples of Proteaceae are yet known in which both two-pored and three-pored pollens occur in one genus or in one species (dimorphic pollen).

In the material examined, *Embothrium coccineum* Forst., the dividing spore mother cells invariably show the decussate arrangement of the young spores in the tetrad (Figs. 11 and 12), and it is clear that the two germ-pores must arise one at each end of the ovoid cells, as the latter undergo very little change of shape when increasing in size to become the mature ovoid pollen grains. (Fig. 13.)

Wodehouse (1932) in a short description, accompanied by a beautiful figure of the two-pored pollen grain of *Banksia Candolleana* Meisn. remarks, " It appears that this grotesque form is firmly established in the family, and is not due merely to some individual or specific anomaly in the arrangement of the pollen cells in their tetrads as the form suggests. Its origin in relation to tetrad formation certainly deserves further study." Now that such a study has been made and it is possible to compare it with the development of three-pored pollen, it appears that the form of the grain in biporose pollen is much more closely bound up with the arrangement of the spores in the tetrad than Wodehouse had supposed it to be.

Although Embothrium is the only genus so far studied, it appears probable that all biporose pollen of Proteaceae will have the decussate arrangement of the spores in the tetrad, as opposed to the tetrahedral grouping found to precede the formation of triporose grains.

As a sequel to this paper, the writer has in preparation a detailed account of the pollen morphology of many of the genera and species of South African Proteaceae.

My grateful thanks are due to Baron Aberconway, C.B.E., who allowed me to collect developmental stages of Embothrium from the plants growing in the open on his estate at Bodnant, Tal-y-Cafn, North Wales, and to Prof. R. H. Compton, who provided developmental stages and fresh material of Leucadendron.

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